Case Report Rapport de cas

Epizootic hemorrhagic disease in a yak

Sarah M. Raabis, Stacey R. Byers, Sushan Han, Robert J. Callan

Abstract — Epizootic hemorrhagic disease virus (EHDV) infection was diagnosed in a 3-year-old yak. The yak had signs of intermittent tremors, dysphagia, oral ulcerative lesions, hemorrhagic enteritis, tachypnea, and thrombocytopenia. Postmortem diagnostics confirmed EHDV (serotype 2) using reverse-transcriptase polymerase chain reaction (RT-PCR). Gross and histopathological results were consistent with EHDV reported in other species.

Résumé — Épizootie hémorragique chez un yack. Une infection par le virus de la maladie épizootique hémorragique du cerf a été diagnostiquée chez un yack âgé de 3 ans. Le yack présentait des signes de tremblements intermittents, de dysphagie, de lésions ulcératives buccales, d'entérite hémorragique, de tachypnée et de thrombocytopénie. Le diagnostic postmortem a confirmé l'épizootie hémorragique (sérotype 2) à l'aide de la technique RT-PC. Les résultats bruts et histopathologiques étaient conformes à l'épizootie hémorragique signalée chez d'autres espèces.

(Traduit par Isabelle Vallières)

Can Vet J 2014;55:369–372

During the summer and early fall of 2012, eastern and mid-western United States laboratories reported an increased incidence of epizootic hemorrhagic disease (EHD) in white-tailed deer. This is an economically important disease in white-tailed deer; however, the effects of EHDV on exotic ruminant hosts have not been completely described. This is apparently the first case report of EHDV in a yak *(Bos grunniens)* that includes clinical presentation, clinical pathology findings, and postmortem examination results.

Case description

A 3-year-old, intact male yak, weighing approximately 300 kg, was examined by the Livestock Service at the Colorado State University (CSU) Veterinary Teaching Hospital in August of 2012. The yak was presented with a 3-day history of depression, anorexia, and neurologic behavior that the owner reported as a loss of prehension and difficulty drinking. The patient lived with 7 other yaks on a farm in northeastern Colorado. There were surrounding cattle and equine facilities; however, no physical contact had been observed by the owner. Initial physical examination findings from the referring veterinarian identified an elevated rectal temperature of 39.7°C (normal bovine range: 38°C to 39°C), apparent dysphagia, and focal tremors of the muzzle while attempting to drink.

On arrival, the patient was lethargic, but hyperesthetic to sound and touch, and in sternal recumbency. We also noted intermittent focal facial tremors during our examination. The patient had a body condition score of 4 out of 9, using a beef cattle scale (1). Vital signs were elevated with a rectal temperature of 39.4°C, heart rate of 120 beats/min, and a respiratory rate of 80 breaths/min. For personnel and animal safety reasons, the yak was sedated with xylazine (Tranquived; Vedco, St. Joseph, Missouri, USA), 0.07 mg/kg body weight (BW), IM, followed by an additional dose of 0.08 mg/kg BW, IM, and was transferred to a stall for further evaluation.

On examination the yak had moderate scleral injection and edema of the muzzle with erythema and hyperkeratosis at the dorsum of the nasal planum. Oral examination revealed a foul odor and injected mucous membranes. A 3-cm diameter focal ulcer was noted on the dorsal dental pad. Thoracic auscultation revealed diffusely increased bronchovesicular sounds bilaterally. No rumen contractions were heard on abdominal auscultation. Auscultation of the right abdomen identified decreased intestinal borborygmi. Transrectal palpation revealed an enlarged rumen with no small bowel distension. The feces were scant, dark black, and watery and also contained fresh blood consistent with melena and hematochezia. Based on ocular globe recession and skin tenting, the yak was estimated to be moderately dehydrated (8%). Physical examination findings were suggestive of systemic disease affecting both the respiratory and gastrointestinal systems. Sedation may have affected physical examination findings, most notably gastrointestinal hypomotility; however, given the patient's degree of systemic compromise observed prior to sedation, it was the authors' impression that these effects did not significantly alter the conclusions from the examination.

Department of Clinical Sciences (Raabis, Byers, Callan), and the Department of Microbiology, Immunology, and Pathology (Han), College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado 80523-1620, USA.

Address all correspondence to Dr. Stacey Byers; e-mail: srbyers@colostate.edu

Blood was collected from the jugular vein for a complete blood (cell) count (CBC) and serum chemistry profile analysis. Using reference values from a study conducted at The Zoological Society of London on clinically normal adult yaks that had been sedated with xylazine, the results of the CBC showed an inflammatory leukogram with a left shift (2). Total nucleated cells were 16.6 $\times$ $10^3/\mu L$ [reference interval (RI): 2.6 to 9.1 $\times$ $10^3/\mu L$], band neutrophils were 1.3 $\times$ $10^3/\mu L$ (RI: 0 to 0.4 $\times$ $10^3/\mu L$), segmented neutrophils were 9.5 $\times$ $10^3/\mu L$ (RI: 1.8 to 3.7 $\times$ $10^3/\mu L$), lymphocytes were 5.1 $\times$ $10^3/\mu L$ (RI: 1.05 to 3.21 $\times$ $10^3/\mu L$), and monocytes were 0.7 $\times$ $10^3/\mu L$ (RI: $\leq$ 0.5 $\times$ $10^3/\mu L$) (2). Thrombocytopenia was also present, with 61 $\times$ 10^3 platelets/μL (RI: 136 to 364 $\times$ $10^3/\mu L$). The patient's PCV was 47% (RI: 24% to 38%) with a plasma protein concentration of 61 g/L (bovine RI: 70 to 100 g/L; RI from CSU Clinical Pathology Laboratory). The fibrinogen was normal at 11.8 μmol/L (bovine RI: 5.9 to 17.6 μmol/L; bovine RI from CSU Clinical Pathology Laboratory). These values indicated significant hypovolemia with presumptive protein loss, as well as inflammation, evidenced by the elevation in band neutrophils.

Bovine reference intervals (CSU Clinical Pathology Laboratory) were utilized in our analysis of the chemistry profile. Results showed azotemia (creatinine 283 μmol/L, RI: 53 to 88 μmol/L), hypoproteinemia (59 g/L, RI: 63 to 88 g/L) secondary to hypoalbuminemia (25 g/L, RI: 28 to 42 g/L), elevated creatine kinase concentration (6680 U/L, RI: 55 to 335 U/L), elevated aspartate aminotransferase concentration (201 U/L, RI: 40 to 140 U/L), hypochloremia (84 mmol/L, RI: 90 to 102 mmol/L), low bicarbonate concentration (13.6 mmol/L, RI: 23 to 33 mmol/L) and an elevated anion gap (37 mmol/L, RI: 13 to 21 mmol/L). The measured strong ion difference [(serum sodium) + (serum potassium) $-$ (serum chloride)] was 47 mmol/L. Blood analysis supported a mixed metabolic acid base disorder consisting of a hypochloremic metabolic alkalosis and a more severe acidosis caused by unmeasured anions such as lactic acid. The hypochloremia was most likely caused by decreased abomasal emptying due to proximal gastrointestinal obstruction or ileus. The high anion gap acidosis was most likely due to elevated lactate secondary to hypovolemia and systemic shock. The azotemia was most likely prerenal secondary to hypovolemia and systemic shock; however, renal disease or urinary obstruction could not be ruled out. The elevated creatine kinase and aspartate aminotransferase concentrations were consistent with muscle damage secondary to prolonged recumbency. The hypoalbuminemia was suspected to be due to gastrointestinal loss based on the clinical findings. An in-house double centrifugation fecal flotation detected few strongyles (10 eggs per gram of feces) and no evidence of coccidia.

The comprehensive problem list for the yak included dehydration; weakness and recumbency; intermittent focal facial tremors; elevated respiratory rate with increased intensity of breath sounds; gastrointestinal signs consisting of oral ulcerations, hypomotility, and scant, watery feces with melena and hematochezia; inflammatory leukogram; thrombocytopenia; azotemia; and a mixed acid base disorder consisting of hypochloremic metabolic alkalosis and high anion gap acidosis. Differentials consistent with these problems included primary neurologic disease (such as rabies, tremorgenic mycotoxin ingestion, septic meningitis); bronchopneumonia with secondary bacteremia (possible pathogens included *Pasteurella multocida, Histophilus somni, Mannheimia hemolytica, Mycoplasma* spp., infectious bovine rhinotracheitis, bovine respiratory syncytial virus, and bovine viral diarrhea virus); primary gastrointestinal disease (such as a bleeding or perforated ulcer, infectious gastroenteritis secondary to bacterial or parasitic infection, enteritis secondary to foreign body obstruction, or peritonitis); anthrax; and vesicular diseases [such as malignant catarrhal fever (MCF), bluetongue (BT), and vesicular stomatitis]. Although unlikely as it is a foreign animal disease, foot-and-mouth disease was also a differential in this case. Epizootic hemorrhagic disease was possible given its clinical similarity to BT and the increased incidence of EHDV cases being reported in white-tailed deer.

Following the initial physical examination, treatment was initiated with tulathromycin (Draxxin; Zoetis, Madison, New Jersey, USA), 2.5 mg/kg BW, SC, and flunixin meglumine (Flunixamine; Zoetis, Florham Park, New Jersey, USA), 1.1 mg/kg BW, IM, for suspected respiratory disease and possible septicemia secondary to enterocolitis. An orogastric tube was passed in order to obtain a rumen fluid sample before administering 9.5 L of oral electrolytes (sodium 60 mmol/L, potassium 38.5 mmol/L, chloride 73.5 mmol/L, acetate 25 mmol/L). Rumen fluid analysis supported our diagnosis of ruminal stasis with approximately 60% of the microorganisms nonmotile or severely hypomotile. The xylazine sedation was reversed with tolazoline hydrochloride (Tolazoline; Lloyd, Shenandoah, Iowa, USA), 4 mg/kg BW, IM. The patient was monitored overnight and remained anorexic, but made attempts to stand and drink water (his total consumption was approximately 0.5 L). He continued to display intermittent lip smacking behavior after swallowing.

The following morning the yak was obtunded and sternally recumbent, but still resistant to handling and examination. He was sedated again with xylazine, 0.16 mg/kg BW, IM. The hair over the right jugular vein was clipped, the skin was aseptically prepared and a 14-gauge catheter (BD Angiocath; Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) was placed in the jugular vein. The yak was started on intravenous fluids. Over the first hour, he was given a 30 mL/kg BW bolus of crystalloids (sodium 140 mmol/L, potassium 5 mmol/L, chloride 115 mmol/L, acetate 30 mmol/L) with 2.5% dextrose and 74 mmol/L of sodium bicarbonate added. After the initial bolus of fluids he was placed on a maintenance rate of 3 mL/kg/h BW, IV. Over the next 2 h, the patient continued to decompensate, became apneic and developed tonic-clonic convulsions. The patient quickly lost consciousness (palpebral and corneal reflexes were absent) and he was humanely euthanized with 60 mL of potassium chloride euthanasia solution IV.

A necropsy was performed at the CSU Diagnostic Laboratory following initial diagnostic testing for rabies and anthrax. Brain tissue was tested for rabies by a direct fluorescent antibody test and was negative. A blood sample was tested by real time PCR and found to be negative for anthrax. Gross necropsy findings included severe diffuse hemorrhagic enterocolitis, moderate

pulmonary congestion, and multifocal to coalescing ulcerative stomatitis. Histopathologic findings showed oral ulcers with multifocally extensive loss of squamous epithelium with minimal inflammatory response, but extensive submucosal hemorrhage associated with necrotizing vasculitis and fibrinoid degeneration of multiple submucosal blood vessels. The sections of lung had moderate interlobular edema and congestion. The intestinal sections showed intraluminal hemorrhage, but cellular details were obscured by marked autolysis.

Given the late summer presentation with periodic regional BT outbreaks and a significant concurrent regional EHDV outbreak in white-tailed deer, we tested for the relevant orbiviruses using a reverse-transcriptase (RT)-PCR on fresh lung for both BTV and EHDV. The yak was negative for BTV but positive for EHDV (serotype 2). An ovine herpesvirus type 2 PCR was conducted on lung tissue to detect sheep associated MCF and was negative.

Discussion

Epizootic hemorrhagic disease virus is an endemic orbivirus that affects wild and domestic ruminants, more specifically white-tailed deer, mule deer, and pronghorn antelope in the United States (3). The EHD virus was of particular concern during 2012, in part due to environmental conditions that favored the vector, *Culicoides sonorensis.* During the late summer and early fall, state veterinary laboratories from Wyoming and Colorado to the east coast were reporting an increased incidence of EHDV infection in white-tailed deer and cattle.

In studies involving experimental infection of white-tailed deer, EHD viremia was found to peak within 6 days (4) with viral replication probably occurring in mononuclear cells and red blood cells (5,6). Subsequently the virus replicates in the vascular endothelium (7), leading to vascular damage and thrombosis with disseminated intravascular coagulopathy in some cases (8,9). It is suspected that the variation in clinical disease seen with infected ruminant species is due to the extent of endothelial damage (8,9). Clinical signs of EHDV infections have been described in white-tailed deer, pronghorn, and bighorn sheep and range from sudden death to chronic disease (3,10). Affected animals may show signs of anorexia, weakness or lethargy, dyspnea, edema of the head and neck, hyperemia (conjunctiva, oral and nasal mucosa, and skin), excessive salivation and nasal discharge, oral ulcers or erosions, lameness, and hemorrhagic diarrhea (3,10–14). Animals may be hyperthermic and become hypothermic prior to death (13). Gross necropsy findings have included petechial and ecchymotic hemorrhages in most viscera, but especially in gastrointestinal mucosa, epicardium, endocardium, papillary muscles, and the lungs (3,10,11,13,15,16). Histological findings of affected tissues include congestion, hemorrhage, thrombosis, and necrosis (3,10,13,15,16).

The clinical findings in the yak in this report were comparable to disease seen in white-tailed deer and in bighorn sheep. Interestingly, the neurologic signs (head tremors and excessive lip smacking) noted in this yak do not appear to have been previously described in association with EHDV. The dysphagia noted is comparable to the excess salivation found in white-tailed deer and is most likely secondary to decreased swallowing due to pain from oral mucosal erosions and ulcerations.

An acute episode of fatal hemorrhagic disease was suspected in a yak in the San Diego Zoo in 1970; however, testing was not performed to confirm EHDV (17). The yak became acutely depressed and anorectic and physical examination showed blood in his feces, sanguineous ocular secretions, and hemorrhagic sclera (17). The necropsy results showed diffuse petechial to ecchymotic hemorrhages on the epicardium and endocardium of the heart, splenic capsule, thymus, lymph nodes, adrenal glands, and the mucosal layer of the abomasum and rumen. There was extensive transmural small intestinal hemorrhage and the lumen contained a large amount of blood. The authors also noted pulmonary edema and congestion (17). The yak in this report had similar findings of visceral and enteric hemorrhage as well as pulmonary congestion and edema. In addition, oral and enteric mucosal ulceration with vasculitis, as detected in this case, is a consistent finding with orbivirus infection in most ruminants (3).

There is currently no specific treatment for EHD in ruminant species. Supportive care, involving anti-inflammatory medications to decrease the inflammatory cascade and antibiotics to prevent secondary infections may be attempted. Prevention is focused on vector control by removing *Culicoides* breeding sites from farms, providing protective housing, and applying pyrethroids to livestock (10). Epizootic hemorrhagic disease appears to rarely affect domesticated ruminants; therefore, not much is known about the disease and its manifestation, especially in exotic species such as the yak. Even in deer, there is still a lack of knowledge concerning the epidemiologic role of EHD in terms of host susceptibility, serotype virulence, and vector capacities (10). In part, this is due to the epizootic nature of the disease, the influence of climate on vector survival, and the introduction of naïve species, such as the yak, into endemic environments. Further studies are needed in host species and maintenance vectors to determine the effects on livestock (both domestic and exotic) and the susceptibility of naïve hosts as the virus continues to modify its geographical distribution. CVJ

References

1. Body condition scoring of beef and dairy animals. Whittier JC, Steevens B: University of Missouri Extension [updated February 9, 2007]. Available from: http://www.thebeefsite.com/articles/906/body-condition-scoring-of-beef-and-dairy-animals Last accessed January 7, 2014.
2. Hawkey C, Ashton D, Hart M, Cindery R, Jones D. Normal and clinical hematology in the yak *(Bos grunniens)*. Res Vet Sci 1983;34:31–36.
3. Howerth E, Stallknecht D, Kirkland P. Bluetongue, epizootic hemorrhagic disease, and other orbivirus-related diseases. In: Williams ES, Barker IK, eds. Infectious Diseases of Wild Mammals. 3rd ed. Ames, Iowa: Iowa State University Press, 2001:77–97.
4. Quist C, Howerth E, Stallknecht D, Brown J, Pisell T, Nettles V. Host defense responses associated with experimental hemorrhagic disease in white-tailed deer. J Wildl Dis 1997;33:584–599.
5. Hoff G, Trainer D. Experimental infection in North American elk with epizootic hemorrhagic disease virus. J Wildl Dis 1973;9:129–132.
6. Stallknecht D, Howerth E, Kellogg M, Quist C, Pisell T. In vitro replication of epizootic hemorrhagic disease and bluetongue viruses in white-tailed deer peripheral blood mononuclear cells and virus-cell association during in vivo infections. J Wildl Dis 1997;33:574–583.
7. Tsai K, Karstad L. The pathogenesis of epizootic hemorrhagic disease of deer: An electron microscopic study. Am J Pathol 1973;70:379–400.

8. Howerth E, Tyler D. Experimentally induced bluetongue virus infection in white-tailed deer: Ultrastructural findings. Am J Vet Res 1988; 49:1914–1922.
9. Howerth E, Greene C, Prestwood A. Experimentally induced bluetongue virus infection in white-tailed deer: Coagulation, clinical pathologic, and gross pathologic changes. Am J Vet Res 1988;49:1906–1913.
10. Savini G, Afonso A, Mellor P, et al. Epizootic heamorragic disease. Res Vet Sci 2011;91:1–17.
11. Roughton R. An outbreak of a hemorrhagic disease in white-tailed deer in Kentucky. J Wildl Dis 1975;11:177–186.
12. Pirtle E, Layton J. Epizootic hemorrhagic disease in white-tailed deer. Characteristics of the South Dakota strain of virus. Am J Vet Res 1961; 22:104–108.
13. Fletch A, Karstad L. Studies on the pathogenesis of experimental epizootic hemorrhagic disease of white-tailed deer. Can J Comp Med 1971; 35:224–229.
14. Fischer J, Hansen L, Turk J, Miller M, Fales W, Gosser H. An epizootic of hemorrhagic disease in white-tailed deer *(Odocoileus virginianus)* in Missouri: Necropsy findings and population impact. J Wildl Dis 1995; 31:30–36.
15. Noon T, Wesche S, Cagle D, et al. Hemorrhagic disease in bighorn sheep in Arizona. J Wildl Dis 2002;38:172–176.
16. Noon T, Wesche S, Heffelfinger J, Fuller A, Bradley G, Reggiardo C. Hemorrhagic disease in deer in Arizona. J Wildl Dis 2002;38:177–181.
17. Griner LA, Nelson LS. Hemorrhagic disease in exotic ruminants in a zoo. J Am Vet Med Assoc 1970;157:600–603.

Book Review
Compte rendu de livre

Pathologic Basis of Veterinary Disease, 5th edition

Zachary JF, McGavin MD. Elsevier, St. Louis, Missouri, USA, 1344 pp. ISBN 9780-3230-7533-6. $165.00.

The 5th edition of Pathologic Basis of Veterinary Disease is an excellent reference for veterinary students and veterinarians who are interested in reviewing the pathophysiology of the diseases observed in practice.

This book provides in-depth coverage in two sections, general pathology and pathophysiology of organ systems, in an exceptionally well-organized resource. The first section covers the normal cell, and the responses of cells, tissues, and organs to injury and infection, as well as the most current methods of studying disease mechanisms, genesis, and progression. There are chapters that review immunology and diseases of immunity, cellular and molecular biology, as well as neoplasia and tumor biology.

The second section covers the alimentary system, including the peritoneum, omentum, mesentery, and peritoneal cavity, the hepatobiliary system and exocrine pancreas, the respiratory system, mediastinum and pleura, the cardiovascular and lymphatic systems, the urinary system, the endocrine system, the bone marrow, and blood cells, the nervous system, skeletal muscle, bones, joints, tendons, and ligaments, the integument, the female and male reproductive systems, the mammary gland, the ear, and the eye.

Additions to this edition include not only the genetic basis of disease, but diseases of the ear, ligaments and tendons, and a new chapter on the mechanisms of microbial infections.

An enhanced website is included with all of the books' images, plus additional images and schematic illustrations that supplement the disease processes discussed in the book. In addition to guidelines for performing a complete, systematic necropsy, and appropriate sample acquisition for selected organ systems, there is a glossary of terms, and methods for gross specimen photography and photomicrography. A very valuable aspect of the website is the availability of all of the book's selected readings, which are also linked to original abstracts on PubMed. The printed book directs the reader to the website when there is additional information available.

The book is extremely well-written, and very easy and enjoyable to read. The authors include interesting historical information and basic clinical information that makes the book both interesting and clinically relevant. The explanations of disease mechanisms are clear and up-to-date.

There are numerous and excellent images and photomicrographs, in addition to full-color illustrations, tables, flowcharts, and diagrams that are extremely well presented and help simplify difficult concepts.

Coverage of World Organization for Animal Health (OIE) reportable diseases is available and adds very important and pertinent information on microorganisms that have catastrophic impact on livestock health and production.

While reading through the list of contributing authors, I was very happy and proud to see that many of the authors are Canadian and experts in their field.

Although the website is a wonderful resource, the only "negative" comment I have regarding this book is that it was frustrating and inconvenient at times to have to put the text aside in order to turn on the computer to obtain further information.

Pathologic Basis of Veterinary Disease is a wonderful tool for veterinary students; I wish I had had this reference while in vet school! There is no question that I will continue to refer to this book for my personal use as a clinician, but I will also use it, particularly the beautiful illustrations and schematics, for teaching undergraduate and graduate students.

Reviewed by ***Lisa Carioto, DVM, DVSc, Diplomate ACVIM,*** *Clinician in Small Animal Internal Medicine and Cardiology, Faculté de Médecine Vétérinaire, Université de Montréal, St. Hyacinthe, Québec.*